Short- and long-term consequences of food resources on Ural owl *Strix uralensis* reproduction

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Academic dissertation

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To Mathilda, Edvin and Lucas

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This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Brommer, J. E., Karell, P., Painter, J. N., Primmer, C. R. & Pietiäinen, H. 2003: Ural owl sex allocation and parental investment under poor food conditions. – Oecologia 137: 140–147.

II Brommer, J. E., Karell, P. & Pietiäinen, H. 2004: Supplementary fed Ural owls increase their reproductive output with a one year time lag. – Oecologia 139: 354–358.

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Summary

1. Introduction

"The optimal animal, born with some amount of energy, proceeds through life gaining and expending energy according to some schedule that maximizes its total reproductive output" (T. W. Schoener 1971)

It lies in every living organism's evolutionary interest to produce as many copies of its genes as possible to the next generation. Although natural selection would favour a maximisation of both reproductive output and life-span, such a combination (so-called Darwinian daemons) can not be achieved in any living organism. According to life-history theory the reason for the fact that not all traits can be maximised simultaneously is that different traits compete with each other for resources. These relationships between traits that constrain the simultaneous evolution of two or more traits are called trade-offs (Stearns 1992, Roff 1992). Trade-offs at different stages of the life of animals have shaped the tremendous variety of life-histories characteristic for different species. In addition, there is also variability among individuals within a species in life-history traits such as age at maturity, number of reproductive events, number and size of offspring, and life-span. In my thesis, these trade-offs are in a very central position.

Trade-offs can in theory occur between any life-history traits (Stearns 1992), but only if the traits considered are resource limited (Martin 1987). Two important trade-offs that are dealt with in this thesis, are between current and future reproduction (a. k. a. the cost of reproduction *sensu* Williams 1966) and between number and quality of offspring (*sensu* Lack 1954). The cost of reproduction is a consequence of current reproductive effort and will reduce the resources available for future reproduction. The trade-off between number and quality (size) of offspring is often referred to as a derivative of the 'Lack clutch' (the clutch that produces most fledglings, Lack 1954), where the trade-off lies in the allocation decision between investment of reproductive resources in fewer high quality offspring or more but less viable offspring (Stearns 1992).

On a phenotypic level trade-offs are considered to be physiological allocation decisions between investment in growth, reproduction or self-maintenance (Williams 1966). The physiological allocation of resources to one trait over the other is a consequence of competition between these traits for limiting resources (Levins 1968). Therefore, allocation of resources to one trait inevitably reduces the resources available for another trait. Hence, individuals that maximise their lifetime reproductive success by optimising allocation of resources to fitness-related life-history traits in given environments should be favoured by selection (Williams 1966). There are, however, caveats to this physiological allocation theory that may weaken the trade-offs: When environmental conditions are favourable and organisms are not stressed or re-

source limited, the costs of allocation of resources to a life-history trait may be minimal or non-existent (Tuomi et al. 1983). Therefore, studies of life-history trade-offs on a phenotypic level, such as the costs of current reproductive effort on future fecundity or survival, benefit markedly from estimates of environmental quality that may alter the strength of the postulated trade-off.

1.1. Reproduction in a variable environment

The food conditions an individual experiences dictate the relative proportion of resources an individual is able to allocate to reproduction (Martin 1987). In a variable environment individuals may allocate resources to reproduction differently if such behaviour enhances the quality of offspring and therefore the fitness of the parents (Roff 1992). This phenotypic plasticity declares that a given genotype or individual does not have a fixed number of offspring it produces, but that the number may increase with experience or vary according to environmental conditions (e. g. Partridge 1989, Stearns 1992). Theoretical models suggest that increased reproductive effort is selected for when conditions are favourable and improving (Hirshfield & Tinkle 1975, Brommer et al. 2000). It is therefore of particular interest in evolutionary ecology to investigate the plasticity of life-history traits and how trade-offs are solved in variable environments.

One cause of environmental variability is cyclic population fluctuations. Cyclic population fluctuations are commonly found in the northern hemisphere and have attracted a great deal of research to examine the evolutionary and dynamical causes and consequences of these cyclic populations (Lindström et al. 2001). Cyclic population dynamics have been described for several organisms in the boreal zone, including voles (Hanski et al. 1991, Norrdahl 1995), snowshoe hares (Krebs et al. 1995) and grouse (Lindström et al. 1996). These cyclic populations form an important part in the ecological communities in the boreal zone, because they affect the reproduction and survival of both avian (e. g. Pietiäinen 1989, Korpimäki 1992, Brommer et al. 2002a, Hakkarainen et al. 2002, Ranta et al. 2003, Sundell et al. 2004) and mammalian predators (Boutin et al. 1995, O'Donoghue et al. 1997). The Fennoscandian vole cycle is thought to be regulated by the synergetic predation effects of their (mainly mammalian) predators (Hanski & Korpimäki 1995, Korpimäki et al. 2002, 2005a, b, Sundell 2006). The vole abundance fluctuates in a three year cycle (low, increase and decrease phase) in southern Fennoscandia (Sundell et al. 2004). The vole cycle thus creates a highly variable and fairly predictable environment for the predatory species that feed on small mammals in the boreal zone and creates an attractive context in which to study the effects of resource limitation on a predator's reproductive strategy in a variable environment. Indeed, the knowledge of reproductive strategies in variable environments in nature is to a large extent thanks to the numerous studies of avian predators that depend on the drastically cyclic prey population fluctuations of voles (e. g. Pietiäinen 1989, Korpimäki & Hakkarainen 1991, Korpimäki 1992, Korpimäki & Wiehn 1998, Brommer 2001, Brommer et al. 1998, 2002a, b). However, in order to understand how a variable environment acts on reproductive traits and survival (fitness), the effects of variable food conditions need to be tested experimentally.

1.2. Reproductive costs in real life: empirical studies and underlying mechanisms

To evaluate the importance of trade-offs and their occurrence in nature, one has to find out to what extent the traits in question are resource limited. Empirically, the cost of reproduction can be tested experimentally in nature in two ways: by manipulation of resource levels during breeding, or by manipulating reproductive effort in a given environment. Experimental studies of birds have improved our knowledge of the cost of reproduction. We know from brood size manipulation experiments in birds that reproductive effort can be negatively associated with parental condition, current reproductive success, survival and future fecundity (reviewed in Lessels 1991). Food supplementation experiments, in which resource levels at breeding is manipulated have given further insight into effects of resource limitation on current reproduction (Martin 1987), and also into resource allocation decisions made by the parents (e. g. Wiehn & Korpimäki 1997, Dawson & Bortolotti 2002).

On a phenotypic level life-history trade-offs are thought to be based on physiological allocation decisions between growth, self-maintenance and reproduction (Fisher 1930, Williams 1966). Within this framework the ultimate goal has been to explore the mechanisms underlying life-history evolution by identifying physiologically functional interactions among various life-history traits (Zera & Harshmann 2000). In the context of physiological trade-offs, parasites and pathogens that alter the resource allocation of individuals have become serious candidates as the mediators of trade-offs (Sheldon & Verhulst 1996, Norris & Evans 2000). This has not always been the case, as originally it was thought that parasites would benefit from prolonging a host's life, and therefore parasites would evolve to do as little harm as possible (Ewald 1983). However, theoretical models revealed that parasites indeed can evolve to be virulent and infer serious damage to the host, and that the host evolves mechanisms to resist these fitness reducing effects of the parasite (Toft & Karter 1990). The theory of parasite-induced costly immune defences got its foothold in the beginning of the 1980s when W. D. Hamilton proposed that parasites may be important both in the evolution of sex (Hamilton 1980) and in sexual selection (Hamilton & Zuk 1982). Hamilton's ideas inspired evolutionary biologists to explore the role of parasites for sexual ornamentation and later to address the same question in a life-history context (Loye & Zuk 1991). By combining the knowledge of reproductive costs in reproductive trade-offs with underlying physiological measurements of immune defence against parasites, a new era of ecological immunology begun its advance into the evolutionary biology literature (Festa-Bianchet 1989, Gustafsson et al. 1994, Norris et al. 1994, Richner et al. 1995, Ots & Hõrak 1996), and new more sophisticated methods to measure immune defence showed that there may well be a direct trade-off between immune function and life-history traits (Deerenberg et al. 1997, Saino et al. 1997, Nordling et al. 1998, Ilmonen et al. 2000, Råberg et al. 2000, Soler et al. 2003, Brommer 2004). However, despite the enormous amount of papers published on functional and evolutionary aspects of ecological immunology, the long-term consequences of immune defence on future life-history components are poorly understood.

1.3. Costs and trade-offs during early development

Parents can affect the phenotypic quality of an offspring under several stages of its development (Lindström 1999). Transgenerational effects where a mother affects the phenotype of its offspring is considered to be of great importance in evolutionary ecology as it may be an important way for a mother to improve the fitness prospects of its offspring (Mousseau & Fox 1998). In altricial birds, the offspring phenotype begins to take form within the mother, develops in the egg with maternally derived resources, hatches in an environment where it needs care from its parents, and develops into an independent juvenile after a period of parental care. Clearly, resource limitation under different stages of development has the potential to modify the phenotypic end product after this cascade of events, which can have impacts on fitness.

Research on environmental factors affecting offspring viability and fitness prospects is a classical theme in evolutionary ecology (Martin 1987, Newton 1989). The early work by Lack (1954) on the potential trade-off between offspring number and quality attracted a lot of research on egg size as a maternally derived measure of offspring quality (reviewed in Martin 1987, Williams 1994, Christians 2002). More recently, the incorporation of knowledge on physiology in egg development achieved in poultry studies (ecological reviews in e. g. Gill 1990, Grindstaff 2003), has opened a new field of study on the evolutionary significance of maternal resource allocation in eggs. Empirical studies of captive and wild birds have shown that these so-called maternal effects may be of great importance for the offspring and may even be of adaptive nature (e. g. Royle et al. 2001, Saino et al. 2002, Grindstaff et al. 2003, Groothuis et al. 2005).

Immunity transferred from a mother to its egg is considered to be crucial for offspring performance, as hatchlings are entirely dependent on innate and maternally derived immunity at hatching (Klasing & Leschchinsky 1998). Resource limitation may play an important role in such maternal transfer of immunity, as it may constrain the amount of resources a mother can invest in the egg. Furthermore, maternally derived immunity can have important consequences, as it may improve resistance to pathogens in the environment that the mother has encountered (Gasparini et al. 2001), improve the offspring's own mobilisation of its immune system (Pihlaja 2006), and enhance growth by weakening the trade-off between growth and immune function (Soler et al. 2003, Brommer 2004, Pihlaja et al. 2006).

A second phase during offspring development where resource limitation can have serious impacts on offspring viability is during the period of parental care. Food limitation during the nestling period generally impairs offspring growth and survival (e.g. Arcese & Smith 1988, Richner 1992, Wiebe & Bortolotti 1995, Wiehn & Korpimäki 1997, Hipkiss et al. 2002, Gill & Hatch 2002). Development during the nestling period may in turn affect survival during the post-natal period (Overskaug et al. 1999) and have long-term effects on parasite resistance in adulthood (Appleby et al. 1999) and future reproductive success (Appleby et al. 1997).

2. Aims and objectives of the study

My aim with this thesis was to investigate the short- and long-term consequences of resource levels on life-history decisions in the Ural owl. I aimed at documenting how experimentally and naturally varying food resources both before and during breeding affect as many different life-history stages of the Ural owl as possible, both within and between generations. The role of parasites and immune function as proximate determinants of physiological consequences of food and mediators of trade-offs were investigated in this thesis.

In chapter I the aim was to investigate sex allocation decisions of Ural owls in relation to natural food conditions and to evaluate whether sex-specific parental investment could explain a biased population sex ratio. In chapter II I experimentally studied the long-term consequences of reduced costs of reproduction on future fecundity by supplementary feeding during the nestling period. Chapter III is tightly linked to chapter II as it describes the role of parasites and immune defence in mediating the cost of reproduction observed in chapter II. In chapter IV I continued to explore the effects of additional food resources during the nestling period on reproductive allocation, but extended it to include two years of naturally different food conditions. I mainly focused on the within season effects of additional resources on offspring growth and future prospects, and how the parents differently allocate additional food resources to own health between years. Chapter V deals with resource limitation on maternal effects. In particular I wanted to investigate if maternal resources can have transgenerational effects on immune function and offspring viability. I focused on how experimentally increased resources prior to breeding affect the quality of the progeny in terms of maternal transfer of immunity and egg size, and on exploration of the consequences of these maternal effects during the nestling period.

2.1. The predator-prey study system: Ural owls and the vole cycle

The Ural owl is a long-lived, monogamous and site-tenacious bird of prey (Saurola 1987) residing in the Eurasian boreal forests from Sweden to Japan (Saurola 1989). Ural owls are sexually size-dimorphic with females larger than males. The sexual size-dimorphy starts to develop when the chicks are around two weeks old. Adult breeding females weigh 950–1,150g (Pietiäinen & Kolunen 1993) whereas males weigh 600–850g (personal observations, II–IV). Ural owls readily breed in nest boxes and start laying eggs within a relatively wide time window from early March until late April, laying between 1–7 eggs (Pietiäinen 1989, personal observations). The eggs hatch after ca. 32 days of incubation after which a nestling period of 25–32 days follows. Ural owl offspring are not fully developed at fledging and therefore the parents continue to attend to them until late summer in August–September, when they become independent. Male and female Ural owls show, as other forest owls and birds of prey, markedly different roles: males are the main hunters during courtship, prior and during incubation, and they also do most of the hunting during the nestling phase. Females, on the other hand, mainly stay at the nest for incubation, and after hatching they mainly brood and feed the offspring.

Female Ural owls are also the main defenders of the nest, from which the species has earned its aggressive reputation (in Swedish the Ural owl is called *slaguggla*, 'the hitting owl').

Ural owl life-history is intimately entwined with the abundance of its main prey, field and bank voles. The high annual variations in vole abundance follow a three year cyclic pattern in southern Fennoscandia, which creates low, increase and decrease vole phases for the owls (Sundell et al. 2004). This fluctuating prey abundance has a strong impact on Ural owl breeding density, as in bad vole years only ca 10% of the population breeds whereas in good vole years 70–80% of the active territories produce eggs (Pietiäinen 1989, Brommer et al. 2002a). Reproductive output is tightly linked to timing of breeding and is highest in decrease phases of the vole cycle (Pietiäinen 1989, Brommer et al. 2002a). Lifetime reproductive success of female Ural owls depends on the age at the onset of the breeding life span, which in turn depends on the vole phase at hatching (Brommer et al. 1998). Most recruits to the breeding population are produced in the increase vole phase (Brommer et al. 2002b), which may select for higher reproductive effort in this vole phase (Brommer et al. 2000).

Also other prey species are important for successful breeding in Ural owls. During the nestling rearing period a large proportion of the diet brought to the offspring by the parents consists of other prey than field- and bank voles, mainly water voles (*Arvicola terrestris*), but also wood mice (*Apodemus flavicollis*), shrews (*Sorex* spp.), thrushes, small birds, and frogs (Korpimäki & Sulkava 1987). The amount of field- and bank voles in the diet of Ural owls in our study area varies between years according to the natural vole abundance, and also the amount of water voles in the diet varies between years, but in a different pattern (Pietiäinen et al. unbublished data). Water voles are therefore, along with the smaller field- and bank voles, the main prey of Ural owls during the nestling rearing period.

2.2. The host-parasite study system: Ural owls, blood parasites and their blood-sucking vectors

Ural owls host a number of blood parasites belonging to haemosporidians, zooflagellates and nematodes. These avian blood parasites are cosmopolitans and appear in more than 2/3 of the examined avian species (Atkinson & van Riper III 1991). The haemosporidians commonly found in avian blood belong to genera *Leucocytozoon* and avian malarial parasites *Haemoproteus* and *Plasmodium*. These haemosporidian parasites infect blood cells and use them as hosts for production of gametes, i. e. they are intracellular parasites (Campbell 1995). Zooflagellates in avian blood belong to the genus *Trypanosoma* and common nematodes in avian blood are microfilarial worms (Atkinson & van Riper III 1991). Contrary to the intracellular parasites, trypanosomes multiply and swim around in the blood plasma feeding on nutrients, and are therefore intercellular parasites. In our study population of Ural owls the intracellular parasites mainly consist of *Leucocytozoon*, but I have also observed *Haemoproteus* species. Trypanosomes and microfilarial worms are also commonly found in Ural owl blood samples (personal observations). In this project my investigations have exclusively concerned the two most fre-

quently observed blood parasites, intracellular leucocytozoans and intercellular trypanosomes. In another owl species, Tengmalm's owl (*Aegolius funereus*) leucocytozoans and trypanosomes were found to infer different effects on life-history traits in the host (Ilmonen et al. 1999). I have therefore chosen to look at both leucocytozoans and trypanosomes as they may infer differential costs also to Ural owl hosts because of their different life-histories and habits.

Blood-sucking ornithophilic dipterans of the family Simuliidae, commonly known as black flies, serve as vectors for both leucocytozoans and trypanosomes. These black flies are infamous biting pests to birds and mammals, as the female black flies require blood for egg production (Malmqvist et al. 2004). In central Fennoscandia the earliest ornithophilic black flies reach maturity in late May and the major emergence of black flies occurs in June–July and continues throughout August (Ojanen et al. 2002, Adler et al. 2004). Massive attacks of black flies can lead to stress, and occasionally death in owls and diurnal raptors (Hunter et al. 1997, Smith et al. 1998). Leucocytozoans are typically transmitted from the vector to the avian host through infectious bites, when sporozoites are transferred to the host. The leucocytozoans undergo asexual reproduction (schizogony) in hepatocytes of the host and develop into merozoites. During the tissue stage (megaloschizont stage), several species of *Leucocytozoon* have been found to cause inflammatory responses in most major organs, including the liver, lung, heart and brain (Atkinson & van Riper III 1991). Hepatic schizonts eventually rupture and merozoites enter the blood stream and infect red and white blood cells, inside which they turn into gametocytes that radically transform the hosting blood cell. An infected blood cell in the host can not function properly as it is used for producing gametes for the parasite. Gametocytes can be retransmitted from the host to the vector, where sexual reproduction takes place. Only the gametocyte stage is detectable in host blood, and it may take several weeks for leucocytozoans to enter the blood stream after an infectious bite from the vector (Atkinson & van Riper III 1991).

3. Methods

3.1. General field protocol

Ural owls were studied in an area of 1,500 km^2 in Päijät-Häme, southern Finland, where all pairs bred in nest boxes 3–4 km apart. Different sub areas of the whole study area were used for the food supplementation experiments (chapters II–V), whereas in chapter I, nests from the whole study population were used. For logistic reasons we focused on the northern and central part of the study area for the supplementary feeding experiments in 2002 and 2003 (chapters II–IV). This sub area of the population includes approximately ¾ of all territories. For the experimental work in 2004–2006 (chapter V) the whole study area was used, but each year the experimental nests were chosen from different parts of the study area to facilitate the logistics and to ensure that new (not previously fed) nests would be available for experimental work in the following season(s).

Nest boxes were checked in one to two week intervals in order to find nests with incomplete clutches to estimate time of egg laying and to count and measure the eggs. Around the estimated time of hatching the nests were followed up in 1–2 day intervals to record origin (egg) of each individual chick and to estimate the hatching dates. Hatchlings were individually marked with a felt-tip pen and ringed after 2–3 weeks. A DNA sample was taken from each individual nestling by pulling a small cover feather from the scapula or by brachial venipuncture. Female parents were trapped by netting them at the opening of the nest box and male parents by trapping with a swing-door trap attached to the box. Parents were weighed with a 1,500 g Pesola spring balance (5 g accuracy) and their body size was estimated from the length of radius-ulna, measured with a ruler (1 mm accuracy) from the elbow to the carpal joint (Pietiäinen & Kolunen 1993). From both parents (if both could be caught) one capillary of blood (75µl) was drawn into capillary tubes after puncturing the brachial vein.

3.2. Estimation of natural food abundance

Field voles (*Microtus agrestis*) and bank voles (*Clethrionomys glareolus*) are the main prey of Ural owls (e.g. Korpimäki & Sulkava 1987) and their natural abundance can be estimated using snap traps (Myllymäki et al. 1971). In this project voles were trapped bi-annually in early June around the time when Ural owl offspring fledge and early October around the time when offspring become independent. The voles were caught by snap trapping in 33 localities throughout the whole study area. In total, each trapping event consisted of 2,544 trap nights. Vole abundance was expressed as a percentage, the number of field- and bank voles trapped per 100 trap nights.

3.3. Food supplementation

This thesis is mainly based on supplementary feeding experiments conducted during the Ural owl breeding seasons in 2002–2006. Ural owl life history (reproduction, survival of parents and offspring) is intimately entwined with the abundance in their main food supply, voles. Hence, manipulation of food supply in this study system will address the main source of environmental variability. More specifically, the idea was to supplementary feed Ural owl nests at different stages of the breeding process to evaluate to what extent breeding is food-limited. In the experiments food was delivered to the nest and could be divided among the family members in whichever way the parents decided. Therefore, in the supplementary feeding experiments we were able to study which life-history component is prioritised if extra resources become available. To further evaluate the methods and to study the consequences of supplementary food in different natural environments, the experiments were repeated in different years.

Food supplementation during the nestling period (II–IV)
In 2002 and 2003 we supplemented Ural owl nests with food during the nestling period. Supplementary food was delivered to the nests in a three day in-

Figure 1. The hatching of offspring triggers male hunting effort. In the increase vole phase 2005 these four chicks and two hatching eggs had 26 microtine voles to feed on. Picture taken by Hanna-Kaisa Mikkola.

terval when also the chicks were weighed and measured. Control nests were visited with the same day interval to weigh and measure the chicks. The supplementary food was placed in the nest box. Accumulation of surplus food in the nest box is a natural phenomenon in the early part of the nestling period (Pietiäinen & Kolunen 1993, figure 1). Feeding begun when the last egg hatched and the number of hatchlings could be determined. On each visit we supplemented approximately 150 g per chick until the oldest chick was 19 days old. Between day 19 and 25 we supplemented approximately 300 g per chick. The supplementary food was a mixture of laboratory mice, rooster broiler chicken hatchlings and voles. The last supplementary feeding was made when the oldest chick in the nest was 25 days old. Female parents were caught, sampled and measured in mid-incubation period (ca. two weeks after laying) and both male and female parents were caught in mid-nestling period (oldest chick 12–14 days old).

Food supplementation prior to laying (V)

In 2004–2006 we delivered supplementary food to Ural owl nests prior to breeding. This food supplementation prior to breeding is a manipulation of the owls' natural behaviour as in birds of prey males deliver food items to the nest during the pre-laying period to nourish the female into breeding condition (Newton 1979, Meijer et al. 1990). Thus, by adding supplementary food to the nest boxes we simulated improved territory quality and/or male hunting success. Feeding and visiting of control nests started more than one month before estimated egg-laying. Supplementary food, which consisted of dead rooster chicken hatchlings, was delivered to the nests in 5–6 day intervals and control nest boxes were checked with a similar interval to standardise disturbance in both groups. Each feeding consisted of 500 g of rooster chicken, and in case there was food remaining from the previous feeding event it was weighed and removed.

3.4. Estimation of parental feeding effort

I estimated the joint parental feeding effort both in relation to the sex ratio of the brood (chapter I) and as an estimate of parental workload in the supplementary feeding experiments during a six day period in the end of the nestling period (chapters II–IV). Nestlings regurgitate pellets containing indigestible prey remains (mainly bones), and these pellets mix in the sawdust layer in the nest box (figure 2). I collected the sawdust from the nest boxes and sorted the bones from the sawdust. The bones are easily identified and most bones remain unbroken. The species-specific number of unique bones was multiplied with the species-specific average weight (according to Siivonen & Sulkava 1994), which enabled me to calculate estimated prey mass and number delivered by the parents. In the feeding experiments (chapter II–IV) supplementary food consisted to some extent of lab mice and voles. Therefore, we broke the femur bones of the supplementary fed voles to later be able to separate them from the natural prey in the prey remains.

The method of estimating prey delivery rate from the prey remains that we have used in chapters I–IV are conservative and could be defined as minimum estimates of parental prey delivery. Prey remains in the nest box should correspond well to the amount of prey delivered by the parents to the offspring, since in Tengmalm's owls feeding effort estimated from prey remains correlated well with prey delivery observations (Hakkarainen & Korpimäki 1994). Errors in the prey delivery estimates, for example that parents would 'steel' prey remains from the nest boxes when they are hungry, are incidents that inevitably may occur and increase the variation between nests in prey delivery estimates from the prey remains. If such events occur, they should, however, not affect the main results that we achieved in chapters I–IV. Firstly, in our experimental setup (II–IV) we test for a difference in prey delivery rate between fed and control nests. Since there is no reason to believe that there is a systematic error in e.g. 'cleaning' behaviour between the fed and control parents, the

Figure 2. Ural owl nestlings in the nest box. By sorting the prey remains from the sawdust layer on the bottom of the nest box, one can estimate parental prey delivery.

results achieved should not be biased in the wrong direction. Secondly, in II–IV we estimated the prey delivery during a period when the female Ural owls are no longer in the nest box. Hence, the chicks have most probably consumed the food themselves.

3.5. Sex determination of offspring

Ural owl nestlings can not be sexed with 100% accuracy in the field, and in order to be able to estimate the primary sex ratio (of eggs) and any sex-specific differences in the broods all offspring were sexed with molecular methods. Hatchlings 3–4 days old were sampled by pulling a cover feather and smaller ones were sampled by brachial venipuncture. Samples were stored in 96% ethanol. All eggs, which did not hatch after the incubation time of 32 days were opened and their contents screened by eye. Any embryonic structure found in an egg was sampled. DNA was extracted from blood, embryonic tissue or the base of feathers using the Chelex method (chapter I) or salt extraction (Chapters II–V). Fragments of the sex-linked CHD-1 gene were amplified using the 2,550 and 2,718 primers described by Fridolfsson & Ellegren (1999). Touchdown PCR conditions followed those outlined in Fridolfsson & Ellegren (1999). Half of the PCR product was electrophoresed through 2% agarose containing ethidium bromide and visualised under UV light. In female Ural owls there are two bands approximately 650 and 1,100 bases in length, while in males only the 650 base band is present.

3.6. Haematology

Blood samples for haematological blood cell and parasite screening were taken from those parental birds, which were included in the feeding experiments in 2002 and 2003. Immediately after venipuncture a small drop of blood (~10µl) from the capillary tube was smeared and air-dried on a glass slide. Within some hours the glass slides were fixed in absolute ethanol for 10 seconds. The blood smears were later stained with May-Grünewald-Giemsa stain for blood cell counts and intracellular *Leucocytozoon* parasite intensity counts (figure 3).

Using a 1000× magnification the number and types of leucocytes within fields of a total of 10^4 red blood cells were determined. We mostly focused on the two most abundant leucocytes: heterophils and lymphocytes in order to estimate the heterophil : lymphocyte (H / L) ratio. Increased values in the H / L-ratio are associated with infectious diseases and starvation (Ots et al. 1998). Intracellular leucocytozoan parasite intensities were estimated as the number in 10^4 red blood cells.

The blood capillaries were centrifuged within 12 hours after sampling at 5,000 rpm for 5 minutes in order to separate the blood cells from the plasma. Immediately thereafter, the capillaries were inspected for trypanosomes for 5 minutes under light microscope using 400× magnification (Woo 1970). During centrifugation trypanosomes accumulate in the capillary at the edge between the white blood cells and the plasma and can easily be identified and counted in the capillaries. Haematocrit (packed cell volume, pcv), was mea-

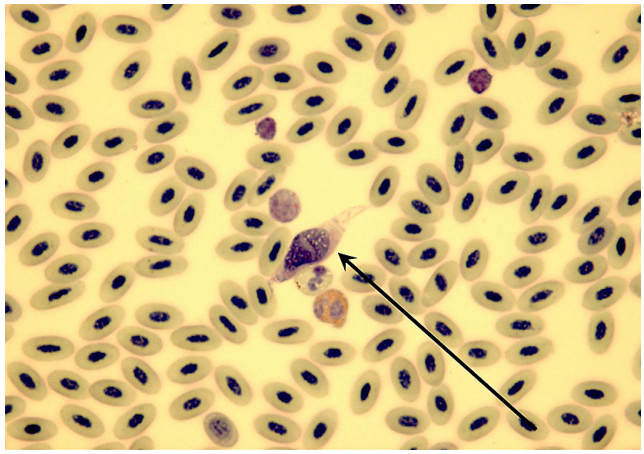

Figure 3. Microscopy picture of a blood smear from a Ural owl. The leucocytozoan parasite (denoted by arrow) is surrounded by different white blood cells.

sured to evaluate any anaemic symptoms, after which plasma was separated from the blood and stored at –18 degrees C.

3.7. Immune responsiveness and immunoglobulin concentration

In the feeding experiment 2003 we measured the humoral immune responsiveness of the female parents to test whether additional resources for breeding enhance immune function (Chapter IV). Immune responsiveness against 2 novel antigens was estimated in 15 fed and 15 control female parents by immunising them intramuscularly in the pectoral muscle with 100µl Diphtheria-Tetanus vaccine (Finnish National Public Health Institute, 7.5 Lf tetanus toxoid and 38 Lf diphteria toxoid, mixed with adjuvant aluminium phosphate at 1.0 mg ml^{-1}). The immunisation took place at the time around hatching prior to which a blood sample was taken from the females. The immune response was measured from a blood sample taken 12 days after immunisation.

In all experimental parent birds (females) in 2003–2006 we measured the general antibody concentrations from blood samples taken when they were caught. In the pre-laying feeding experiment (chapter V) anti body concentration was also measured from offspring at hatching and at fledging. The blood plasma, which was separated from the blood cells in capillary tubes, was used to determine antibody concentrations (Immunoglobulin G) using ELISA (enzyme-linked immunosorbent assay).

4. Results and Discussion

4.1. Which components of avian reproduction are food-limited?

In ecology and evolutionary biology food limitation is a central issue as food influences life-history traits, population sizes, and community structures (Martin 1987, Newton 1998). The effects of food supply on reproduction have been particularly well studied in birds. By supplementing food before egg laying, during incubation and during the nestling stage knowledge has ac-

cumulated on reproductive costs and resource allocation to different life-history components. Food supplementation prior to laying has contributed greatly to our understanding of seasonal reproductive decisions: reproductive output is considered to be an optimisation of the advantages of early breeding to improve offspring survival and the advantages of delayed laying for gathering of parental resources for breeding (Daan et al. 1990, Rowe et al. 1994). Food availability can also affect the sex allocation decisions made by the parents (Komdeur et al. 1997, Appleby et al. 1997, Nager et al. 1999, Byholm et al. 2002), since in vertebrates sex biases are most often environmentally induced (Charnov 1982, Frank 1990).

Food supplementation during the nestling period explicitly tests to which extent parents and offspring are limited by food. Offspring are often food-limited as food supplementation typically increases the quality and/or number of offspring (e.g. Arcese & Smith 1988, Richner 1992, Wiebe & Bortolotti 1994, Wiehn & Korpimäki 1997, Hipkiss et al. 2002, Gill & Hatch 2002). In some species, also the somatic condition of the parents (and most often the female parent) responds positively to food supplementation (e.g. Garcia et al. 1993, Wiehn & Korpimäki 1998, Dawson & Bortolotti 2002). However, food limitation studies have been restricted to encompass only short-term consequences in different stages of the breeding event, such that overall reproductive costs have remained relatively unexplored (but see Gill & Hatch 2002). Furthermore, the mechanisms and physiological effects of these reproductive costs are still poorly known and little is known about transgenerational effects of resource limitation. In the following sections I will discuss my findings in how resource limitation affects life-history decisions in Ural owls.

4.2. Is the larger sex more costly to produce?

In paper I, I studied whether sexual size-dimorphism in Ural owls can infer a sex-specific cost in offspring production, which could select for biased sex ratios in Ural owl broods. Sex ratios at birth should follow a 1:1 ratio because of frequency dependent selection (Fisher 1958). If the population-wide sex ratio differs from parity, the (reproductive) value of the rare sex would be higher and parents would be selected to produce more of the rare sex until parity is restored. However, if the parental investment differs between sons and daughters, the optimal population-wide sex ratio should be inversely related to the investment ratio (Fisher 1958). Sexual size-dimorphism creates such potentials for sex-specific energy demands in offspring (Andersson et al. 1993, Riedstra et al. 1998), which could select for biased Fisherian sex allocation towards the cheaper (smaller) sex. Experimental evidence underlines that food supply is pivotal for sex-allocation decisions in size-dimophic birds. A series of combined manipulations of female condition and food in lesser black-backed gulls (Nager et al. 1999) and great skuas (Kalmbach et al. 2001) shows that size-dimorphic birds overproduce the smaller sex under adverse conditions. Through egg-removal these authors induced females to produce more eggs; supplementary fed females produced eggs at equal sex ratios, whereas control females overproduced the smaller sex.

I found no evidence that Ural owl parents would invest (in terms of food provisioning) more in the larger sex (daughters), although the primary popu-

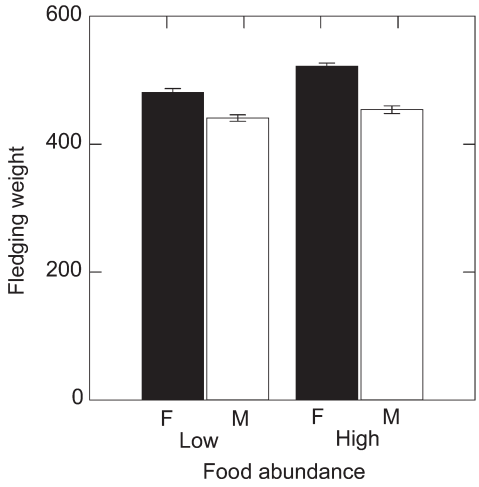

Figure 4. Fledging weight of male (M) and female (F) offspring in poor (Low) and good (High) natural food conditions. From chapter I.

lation sex ratio was significantly biased towards the smaller sex (sons). Food limitation could potentially explain a lack of sex-specific differences in feeding investment, as low parental food provisioning had more pronounced effects on fledging weight in daughters than in sons (figure 4). Such a scenario would indicate that the growth of daughters is more food limited than the growth of sons. In the fourth paper (IV) I analysed the growth of offspring in food supplemented nests and control nests. If female offspring would benefit more from improved food conditions one would expect a larger effect of supplementary food on daughters than sons. Although supplementary feeding had a positive effect on offspring growth I did not find any interactions between sex and food supplementation on offspring growth. Hence, I found no evidence for the hypothesis that daughters would gain more from additional food resources than sons.

Interestingly, I found in chapter V that sons had higher antibody levels than daughters at fledging. Since higher levels of general antibodies estimate immunological 'condition' (Apanius & Nisbet 2006), our result suggests that sons would invest more in immune function than daughters. Development of immunity is considered to be costly (Klasing & Leschchinsky 1998, Lochmiller & Deerenberg 2000) and hence, sons potentially use more resources to development of immune function as they do not grow as big as daughters. As a consequence of such sex-specific investment in growth and immunity, the smaller sex (sons) would not be cheaper to produce than the larger sex (daughters).

4.3. Food limitation on reproducing parents: reproductive costs and carry-over effects

During an individual's breeding life-span allocation of additional resources to own maintenance rather than investment in current offspring growth or survival is controversial as the fitness benefits of improved own maintenance can be jeopardized before the next breeding event. If, however, future prospects for reproduction are good, investment in self-maintenance may create higher

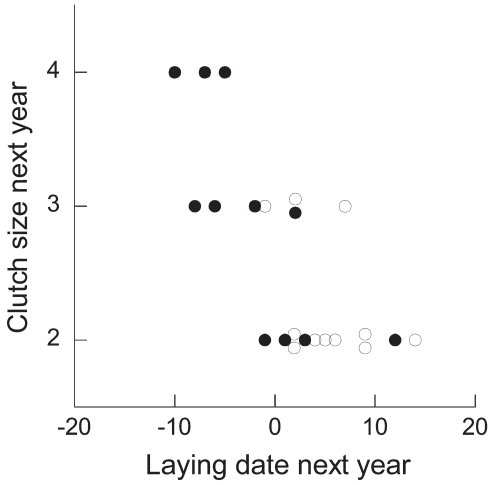

Figure 5. Carry-over effect of food on next year's reproduction. The figure shows the clutch size-laying date relationship in the season that followed the food supplementation experiment. Fed nests are denoted by filled circles and control nests by open circles. For further details see chapter II.

fitness benefits than additional investment in current offspring. According to the terminal investment hypothesis (Clutton-Brock 1982, Pärt et al. 1992), uncertain future forecasts in reproduction may on the other hand select for increased investment in current reproduction. Naturally, the life-history of the organism plays a major role in this life-history tactic: short-lived species with few potential breeding attempts should invest in current reproduction, whereas long-lived species should invest less per breeding event and more in their own survival to ensure a long breeding life-span.

I found that food supplementation during the nestling period had profound effects on parental behaviour in Ural owls, which consequently affected both parental condition and offspring growth. Supplementary food reduced the parental feeding effort (II–IV). Clearly, such a result indicates that a reduction in own parental effort would reduce current costs and have positive effects on residual reproduction. This is exactly what I discovered in the Ural owl: supplementary fed Ural owl pairs bred one week earlier and produced 0.6 eggs larger clutches than control pairs in the following year (figure 5). Hence, the future reproductive component was food-limited. It is striking that a reduction in the breeding costs during a relatively short period of time (the nestling period is 25–32 days long) can have consequences throughout the whole year and affect reproductive output one year later. Similar findings have been discovered in brood size manipulation experiments of collared flycatcher *Ficedula albicollis* (Gustafsson & Sutherland 1988) and blue tits *Cyanistes caeruleus* (Nur 1988), where increased reproductive effort reduces reproductive output in the following year. However, a food supplementation experiment explicitly tests how additional food is divided tactically to maximise reproductive output, whereas experimental brood size enlargement or reduction is a measure of the consequences of a manipulation of the reproductive decision (number of offspring) the parents already have made. The difference between the experiments may seem subtle, but may as well result in a different outcome from a life-history perspective. That is, a brood size manipulation experiment does not mimic a natural situation, in which a parent makes its allocation decision itself, and theoretically the experiments are not synonymous.

In any case, in Ural owls, reproductive costs due to limiting food resources during breeding have the potential to bring about long-term carry-over consequences on future reproduction. This carry-over phenomenon is potentially widely occurring in many other long-lived species. Food supplementation studies in other long-lived birds generally find that parents reduce their feeding effort and have improved body condition (Wiehn & Korpimäki 1997, Dawson & Bortolotti 2002). Potential carry-over effects have, however, remained unaddressed in such studies due to the difficulties in following the individual birds between breeding attempts.

4.4. Can costs of reproduction be mediated by parasites and infectious diseases?

The proximate mechanisms for trade-offs between current and future life-history components are unknown, but have been suggested to be mediated through suppressed immune function and physiological health (Sheldon & Verhulst 1996, Norris & Evans 2000, Schmid-Hempel 2003). It has also been acknowledged that improvement in the physiological health of the parents can have the potential to mediate improved residual reproductive value (Zera & Harschman 2001).

A number of studies have documented that experimentally increased reproductive effort has immediate effects on immune function by impairing parasite resistance (*e.g.* Gustafsson et al. 1994, Norris et al. 1994), and decreasing humoral immune responsiveness against a novel antigen (*e.g.* Deerenberg et al. 1997, Nordling et al. 1998). Only few studies of natural vertebrate populations have been able to explore the link between reproductive effort and immunocompetence, and relate this trade-off to future survival and future fecundity. Such studies have documented a trade-off between immune responsiveness and reproductive effort and related this trade-off to a change in reproductive output in the following year (Hanssen et al. 2005) or survival after the treatment (Ardia et al. 2003, Stjernman et al. 2004). However, to explicitly test whether changes in immune function during breeding can have long-term consequences on immune function *per se*, one needs to explore the changes in immune function also at the time when the costs are paid. Hence, to show that parasites are important mediators of reproductive costs, each component in the chain of events from current reproduction to future fitness benefit should be verified (Norris & Evans 2000, see also Stjernman 2004).

In chapter III, we found that increased resource levels during reproduction affected parasite resistance and immune function. Fed parents reduced their feeding effort, which enabled female parents to invest additional resources in immune defence and resistance against leucocytozoan parasites during breeding instead of allocating resources to chick provisioning.

This improved resistance against the intracellular parasite *Leucocytozoon* had long-lasting carry-over effects to the following breeding season as fed females were less infected in the following year (figure 6a). Therefore, it appears that investment in leucocytozoan parasite resistance is costly, especially under the nestling period when energetic demands for breeding are high. Furthermore, as fed females increased their reproductive output in the year after the experiment, our result clearly implies that costs of reproduction can in-

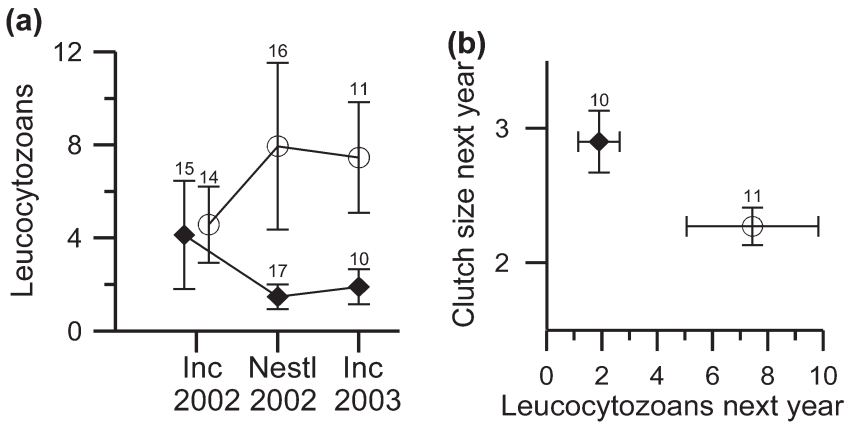

Figure 6. Parasite load in fed (filled) and control (open) females. Given are (panel a), the intensity of *Leucocytozoon* (mean ± SE) before (mid-incubation), during (mid-nestling period 2002) and one year after the food supplementation (mid-incubation 2003). Panel b shows the relationship between next years clutch size (mean ± SE) and leucocytozoan intensity (mean ± SE) in mid-incubation period 2003 for fed and control females. For further details see chapter III.

deed be mediated by parasites (figure 6b). This finding that Leucocytozoan parasites infer large costs to the host during chick provisioning is also supported by other experimental work on wild birds, although the carry-over effects of the costs in these studies have not been possible to evaluate. In Finnish kestrels, supplementary fed female parents reduced their hunting effort and have lower parasitaemia than control females (Wiehn & Korpimäki 1998). Similar results were also found in Tengmalm's owls where food-supplemented females had lower loads of blood parasites compared to control females (Ilmonen et al. 1999). Furthermore, in passerine birds experimentally reduced infections of intracellular parasites *Leucocytozoon* and *Haemoproteus* have been found to improve several reproductive traits within the breeding season (Merino et al 2000, Marzal et al. 2005, Tomás et al. 2006).

Critically, one could argue that the observed decrease in *Leucocytozoon* parasite load in fed Ural owl females (figure 6a) would have been entirely due to a lower encounter rate of the vector (black fly, Diptera: Simuliidae) for *Leucocytozoon*. This is unlikely, as we found a rapid decrease in *Leucocytozoon* parasite load in females (less than 30 days), which occurred before the emergence of the parasite vector. From the time around when the Ural owl offspring fledge (late May) ornithophilic black flies do become extremely numerous all through the summer (Ojanen et al. 2002, Adler et al. 2004). Therefore, leucocytozoan load in Ural owls could very well be influenced by summer abundance of vectors, but in this case it would have occurred after the food treatment that caused the difference in parasite load had ended. Furthermore, we found that the difference in parasite load between fed and control females persisted to the following breeding season, and was hence not affected by the fact that black flies are abundant in summer (figure 6a). It is therefore most likely that leucocytozoan infection can infer a serious cost to the host and that fed Ural owl females had improved parasite resistance compared to control females, which increased their future reproductive output.

4.5. Naturally and experimentally varying resources during the nestling period: consequences for parents and offspring

According to life-history theory, the best option for a parent is to adjust its reproductive effort in order to maintain high survival probability if the environment is unpredictable (Hirshfield & Tinkle 1975). A lower survival probability for juveniles than adults further selects for lower parental effort (Brommer 2000). Hence, a trade-off between parental survival and parental allocation can arise, where the optimal solution depends on the reproductive value of the parents and the reproductive value of the offspring (Lindström 1999, Brommer et al. 2000). Parental care during the nestling period is ubiquitous for production of successful offspring in altricial bird species (e. g. Clutton-Brock 1991). The environmental conditions parents experience during the nestling period determine the amount of effort the parents invest in the offspring, which in turn determines reproductive success (Martin 1987, Newton 1989). Still, the fitness consequences of food resources during the period of parental care (i.e. the nestling period) for offspring growth and fitness prospects, and parental health are poorly known. In paper IV my aim was to compare parental resource allocation to own health and offspring growth in supplementary-fed and control nests under variable natural food conditions, and hence to study which life-history component is prioritised if extra resources become available and whether the allocation decision depended on the vole cycle phase.

Consequences on parental resource allocation and reproductive costs in a variable environment

We supplementary fed Ural owl nests during two reproductive seasons in an increase and a decrease phase of the three-year vole cycle (figure 7). Earlier descriptive studies on the same Ural owl population have found that increase and decrease vole cycle phases provide strongly contrasting future perspectives for both Ural owl parents and their offspring, since parental survival and offspring recruitment is lower after the decrease phase than after the increase phase (Brommer et al. 2002a, Brommer et al. 2002b). Our aim was therefore to investigate whether parents, in order to maximise fitness, differ in their allocation decisions between current reproduction and own maintenance, depending on the phase of the vole cycle.

We found in chapter IV that in both years of the experiment, Ural owl parents halved their joint feeding effort when the nest was food supplemented (figure 8). By reducing the feeding effort Ural owls apparently aimed at reducing their cost of reproduction. However, the consequences of reduction in feeding effort for parental health differed between sexes and between years: food supplementation had no health benefits on males, whereas female immune function and parasite resistance was improved in the increase phase but not in the decrease phase. Similar results have been found in vole-eating Eurasian kestrels (Wiehn & Korpimäki 1998). Interestingly, as discussed above, the improvement in Ural owl female health in the increase vole phase led to increased reproductive output in the following year (II), which appeared to be mediated by improved parasite resistance (III). Such a carry-over effect was not observed after the decrease phase. I propose that the absence of a carry-

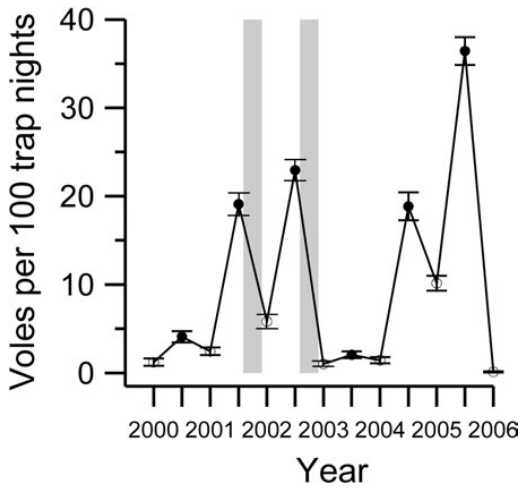

Figure 7. Vole-abundance from 2000 to 2006 forming two vole cycles. Vole abundance in spring is denoted by open circles and vole abundance in autumn by filled circles. The gray areas represent the time period when the supplementary feeding experiments were carried out. A carry-over effect on reproduction was discovered from 2002–03 when vole abundance increased, but not in 2003–04 when vole abundance crashed.

over effect in the decrease phase would be a consequence of the vole cycle (figure 7): Firstly, there were no health benefits of supplementary food in females, which could have mediated the carry-over effect in reproduction. Therefore, females appeared not to be food-limited. Secondly, the voles crashed during the breeding season, which did not allow for successful breeding in the following year. Only two pairs from the experiment bred in the low vole phase in 2004. Any positive effects of supplementary food were overridden by the harsh conditions during the low vole phase. Thirdly, parental survival was low after the vole crash, especially for the food providing males (only 12% survived), possibly because they had to switch to hunting larger prey (see figure 8). Hence, food limitation during the nestling period can in Ural owls have carry-over effects on future reproduction, but such effects are regulated by the drastically varying natural food supply and potentially by differential parental allocation of reproductive effort.

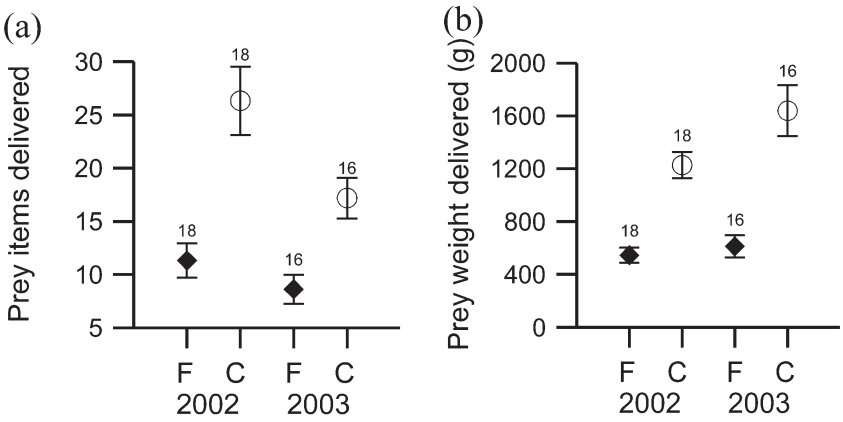

Figure 8. Parental prey item delivery (a) and prey weight delivery (b) between day 19 and 25 of the nestling period in fed (F, filled) and control (C, open) nests in 2002 and 2003. The error bars (sample sizes above) indicate one standard error of the mean.

Consequences on offspring growth and ectoparasite avoidance

In paper IV I also found that offspring benefited from the supplementary food, although parents reduced their feeding effort during the nestling period. We analysed the growth of offspring through the whole nestling period and found that offspring in supplementary fed nests reached higher asymptotic weights than offspring in control nests in both the increase and the decrease vole phase. However, the reduction in parental food provisioning counterbalanced the effects of supplementary food and hence the increase in asymptotic weight was relatively small. Fed offspring were on average 11g heavier, which compared to the asymptote of 544 g in females and 500 g in males is relatively subtle. Even small increases in body mass at fledging can however be beneficial, since in the closely related tawny owl (*Strix aluco*) mortality during the post-fledging period is higher for lighter fledglings (Overskaug et al. 1999). Furthermore, supplementary food not only increased the asymptotic weight of fledglings, but also enabled the offspring to grow faster and hence to fledge earlier. Thus, the age at which offspring were able to fledge was food-limited. Furthermore, higher intensities of black flies (ectoparasites) reduced the time offspring stayed in the nest. The end of the Ural owl nestling period coincides with the beginning of the outbreak in adult black fly numbers in central Fennoscandia (the end of May, Ojanen et al. 2002, see also Adler et al. 2004). Hence, increased resources during growth can help Ural owl offspring to avoid high densities of black flies. Avoidance of black flies can be beneficial for fledglings, since black flies can cause serious stress to young birds and even death (Hunter et al. 1997, Smith et al. 1998). Furthermore, black flies are also vectors for intra- and intercellular blood parasites, including *Leucocytozoon* (see III, figure 3). In general, young birds are most susceptible to intracellular blood parasite infections and most mortality due to infection occurs before adulthood (Atkinson & van Riper III 1991). It has also been experimentally shown that in the presence of ectoparasites offspring grow faster and fledge earlier (Badyaev et al. 2006). Thus, our results imply that in Ural owls a faster growth results in fledging at a younger age, which allow escaping high densities of blood sucking black flies, which rapidly become abundant in the nest at the very end of the nestling period.

4.6. From mother to egg: transgenerational effects of maternal resources on offspring phenotypic quality

Life-history theory predicts that in an unpredictable environment parents adjust their reproductive effort to maintain high survival probability (Hirshfield & Tinkle 1975). The unpredictability of the environment can be further intensified by the time delay between reproductive decisions made by the parents, and the environmental conditions that the offspring actually face when they are born (Lindström 1999). In birds, and many other oviparous organisms (Mousseau & Fox 1998), mothers can compensate for this reduction in offspring quality already before the offspring hatch by improving the quality of the eggs (Martin 1987, Williams 1994, Grindstaff et al. 2003, Groothuis et al. 2006). This buffering of offspring against environmental conditions can be an important component in life-history evolution, but only if the traits involved are resource limited and compete with other traits.

In paper V we investigated how increased resources prior to egg laying can influence the maternal resources allocated to reproduction. Basically, a female has two choices by which she can increase her fitness when resources are increased: an increase in clutch size (Daan et al. 1990) or an increase in quality of the eggs (Williams 1994). We conducted an experiment where food was delivered to Ural owl nests prior to laying. In order to get a general picture of such effects and how different natural food abundance affects maternal effects we replicated the experiment twice. Hence, we were also able to get a hint on potential variations in maternal effects that could be caused by the highly variable food conditions (voles). Emphasis was laid on the transmission of immunological components, as immunological maternal effects have been suggested to be of great importance for offspring viability (Grindstaff et al. 2003, Pihlaja et al. 2006).

Resources and maternal investment in eggs

We found that supplementary food prior to laying increased the body mass and elevated the antibody levels of Ural owl females. As such, fed females had increased resources that they could allocate to reproduction. According to Norris & Evans (2000) elevated antibody concentrations can in principle mean two things: an individual has higher antibody concentrations because it is seriously ill or because it is more immunocompetent. The positive relationship between body mass and antibody concentrations in Ural owl females does suggest that the fed females in this study did have improved immunocompetence (figure 9a, b). Higher general antibody concentrations (IgG, passive immunity) at egg laying thus seem to be an indication of improved immunocompetence in Ural owls (see also Grindstaff et al. 2003, Apanius & Nisbet 2006).

Fed females did not lay larger clutches but instead increased the size of the eggs and transmitted higher antibody concentrations to the eggs compared to control females (figure 9c, d). Our results therefore show that egg size and transmission of antibodies to the offspring are strongly resource limited maternal effects – both egg size and antibody concentrations were increased in all three years (figure 9c, d). Such a strong impact of pre-laying resources on maternal effects suggest that maternal investments are costly to a Ural owl female, and can only be undertaken when increased food resources are at a female's disposition. Hence, we can conclude that maternal effects are food-limited in Ural owls, despite the highly variable natural food conditions (low, increase and decrease phase of the vole cycle).

Consequences of maternal effects on early development

Egg size is an important life-history trait mediating maternal influences on offspring phenotype, because it is simultaneously both a maternal and an offspring character (Mousseau & Fox 1998). An egg is produced by the mother and it determines the initial size and resources of the hatched offspring. In birds, however, egg size is considered to be rather inflexible on the individual level, and relatively little affected by food conditions (Christians 2002). Our results show that Ural owl females are able to respond to improved food conditions by a remarkable increase (up to 16%) in egg size and clearly demonstrate that egg size is a costly and highly plastic trait. Williams (1994) concluded that an increase in egg size may be a small additional cost to the female

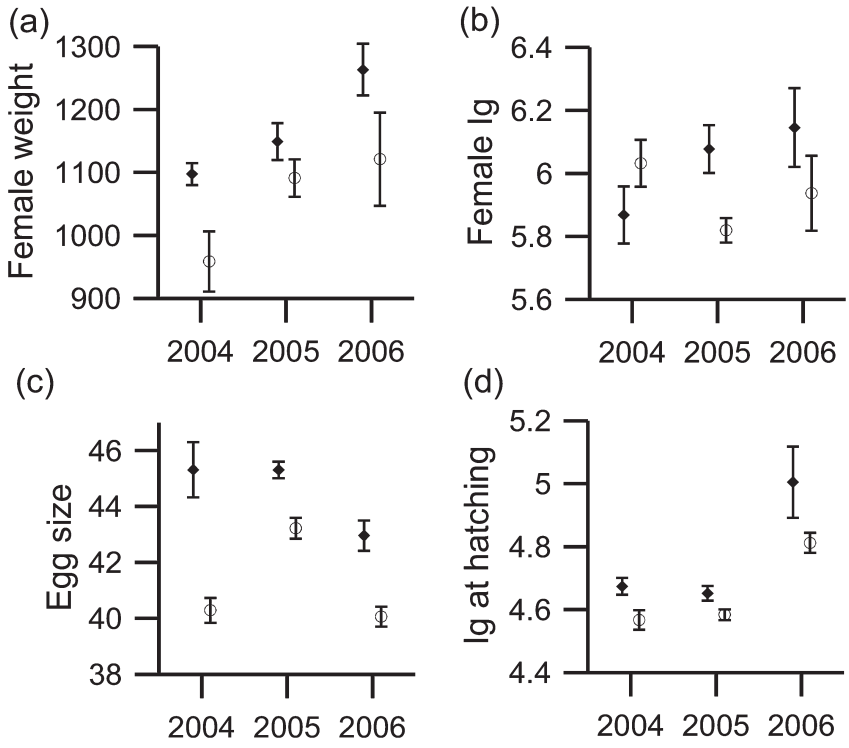

Figure 9. Effects of supplementary food on female size-corrected weight (a), female antibody levels (b), hatchling antibodies (c) and egg size (d). Individuals from the fed group are denoted by filled squares and controls by open circles.

that may be beneficial for the offspring during early development. We found indeed that increased egg size had a strong positive effect on offspring body mass at fledging (3.8 grams heavier at fledging / egg cm^3), indicating that there are clear fitness benefits from developing in a large egg.

Maternally transferred antibodies to offspring may enhance offspring's own antibody production, survival and early growth (Pihlaja et al. 2006, Kilpimaa et al. 2007). We did not find any evidence that increased antibody levels at hatching would improve an offspring's early growth or subsequent antibody production. However, we may have overlooked possible effects of maternal antibodies at hatching, since they may stimulate the mobilisation of other parts of the immune system (Grindstaff et al. 2003) or possibly even affect immune function across multiple generations (Lundin et al. 1999).

5. Conclusions and future prospects

One of the central findings in my thesis is that resource levels during breeding had carry-over consequences on future fecundity, and hence reproduction infers a cost on future reproduction (II, figure 10). The cost of reproduction is one of the cornerstones in the theory of life-history evolution (Williams 1966). Since direct empirical evidence of this phenomenon is rare the contents of this thesis contributes to the knowledge of life-history decisions in nature.

A fascinating addition to our finding of a cost of reproduction on future re-

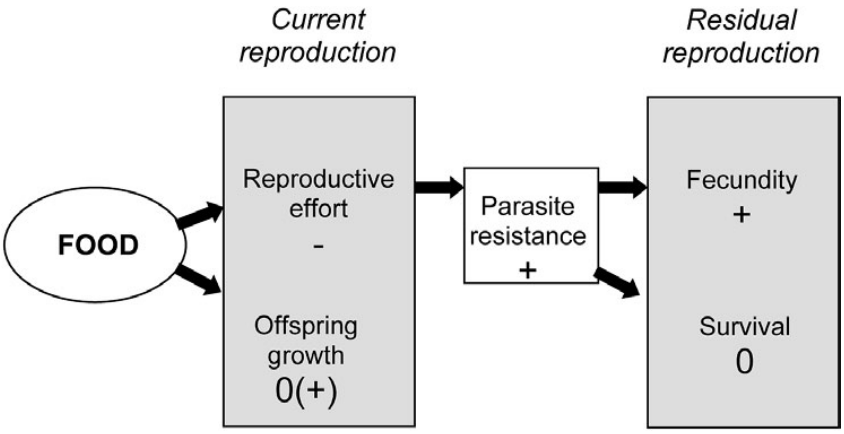

Figure 10. Diagram showing the consequences of supplementary food during the nestling period on current and (female) future reproduction (Summarised results from chapters II & III). Arrows indicate the (postulated) pathway and +, – and 0 denote the direction of the effect.

productive output in nature (II) is that we also found evidence that this cost was mediated by parasite resistance (III, figure 10). Therefore, we have empirical evidence for the trade-off between reproductive effort and immune function, which was proposed by Sheldon & Verhulst (1996) and Lochmiller & Deerenberg (2000). I found that additional resources during breeding reduced parental effort in the current breeding season and subsequently increased reproductive effort in the following season. As a consequence parasite resistance was still improved in the following season, which was coupled with increased fecundity (figure 6). This is to my knowledge the first evidence of the physiological mechanisms underlying the costs of reproduction, where the cause and consequence of both mechanism and the life-history components have been measured. However, to further validate the causality of parasite resistance as a mediator of reproductive costs, future studies should aim at directly manipulating parasite intensities and evaluate the long-term (carry-over) effects on residual reproduction and residual parasite resistance (see also Merino et al. 2000).

I also found that when food is not limiting during the nestling period Ural owl parents reduce their feeding effort regardless of the natural conditions (IV, figure 8), and hence aim at investing in own maintenance (figure 10, mainly female parents, see also Wiehn & Korpimäki 1997). This finding supports the results from long-term observational data that lifetime reproductive success in Ural owl females is mainly determined by their breeding life-span (Saurola 1989, Brommer et al. 1998). It also follows the theoretical expectations that in long-lived species parents should adjust their parental effort to maintain high survival probability (Hirshfield & Tinkle 1975, Stearns 1992). In future studies of food-limitation during reproduction, one should take into account both parental and offspring components of fitness, and evaluate (if possible) the long-term consequences on residual reproduction and survival of the parents. Both parental and offspring components are important to estimate, since adjustment of parental food provisioning, that can have consequences on both parents and offspring, may to a large extent depend on the

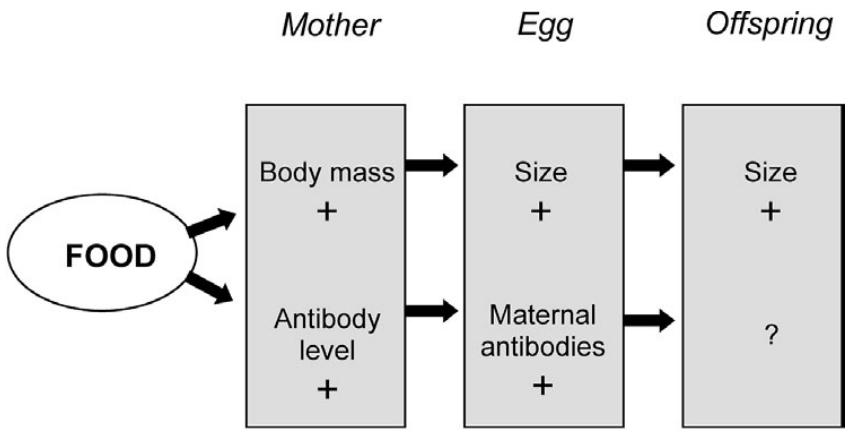

Figure 11. Diagram showing the consequences of supplementary food prior to egg laying on mother, maternal allocation in eggs, and offspring (Summarised result from chapter V). Arrows indicate the (postulated) pathway and +, –, and 0 denote the direction of the effect.

species life-history. For example, in contrast to our findings short-lived species and species with less variable reproductive output (i. e. living in a less variable environment) would not be expected to adjust their reproductive effort. Hence, the result would be that mainly offspring condition would be improved (Arcese & Smith 1988, Richner 1992, Gill & Hatch 2002), or that parents would adjust timing of breeding in the following year (Grieco et al. 2002). Furthermore, carry-over costs of reproduction may be found mainly in so-called capital breeders (*sensu* Drent & Daan 1980) whereas they may be more uncommon in income breeders (*sensu* Drent & Daan 1980). Capital breeders have to acquire their energy reserves well in time before breeding, which can to a larger extent be affected by conditions experienced as far as in the previous year (II). On the other hand, income breeders may make their reproductive decisions more as a function of the prevailing food conditions. Therefore, I would encourage researchers to continue to experimentally investigate the role of food conditions during breeding in various organisms with different life-histories, as it will give valuable information on environmentally induced life-history tactics and will improve our knowledge of both evolutionary and population dynamics.

In chapter V we found that maternal effects are costly in Ural owls. Resource limitation on maternal investment in egg quality (egg size and maternal transfer of antibodies via the egg) in Ural owls occurred in different years with dramatically different natural food abundance. The fitness benefits of increased maternal investment were evident as improved egg quality (egg size) had a clear positive effect on offspring size at fledging (figure 11). Hence, maternal effects may have long-term consequences that prevents the occurrence of life-history trade-offs during early development (e. g. between investment in growth and immunity (Soler et al. 2003, Brommer 2004, Pihlaja 2006). The indirect consequences of maternal effects may, however, have consequences throughout and even beyond the juvenile period.

The main message of this thesis is that food regulates the reproductive decisions of Ural owls. However, there seem to be different short- and long-term

consequences: In chapter II, I found that food resources one year earlier affected the number of eggs a female laid. Also, using long-term descriptive data from the same Ural owl population Brommer et al. (2002a) found that food conditions in autumn prior to the breeding season best explain breeding success. Compared to this, I discovered in chapter V that quality of each egg was determined shortly prior to laying. The long-term effects of food may be related to the findings that owls have low energy metabolism and expenditure compared to other birds of similar size (Wijnandts 1984, Weathers et al. 2001). Hence, in large owls (capital breeders *sensu* Drent & Daan 1980), such as the Ural owl, resource allocation decisions on whether to breed and how many eggs to produce need to be made already several weeks before the actual breeding (Hirons et al. 1984). Shortly prior to breeding the owls may be able to regulate the final amount of resources put in each egg, and potentially even reabsorb eggs if the conditions become too adverse. Hence, as the final words of this summary I would like to conclude that in Ural owls the number of offspring is a long-term consequence of food, whereas quality of offspring is a short-term consequence of food. Such different consequences of resource availability may be important in theoretical models of population and evolutionary dynamics.

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